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Research Article

Oxygen Concentration-Dependent Oxidative Stress Levels in Rats

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Introduction. We determined derivatives of reactive oxygen metabolites (dROMs) as an index of oxidative stress level (oxidant capacity) and biochemical antioxidant potential (BAP) as an index of antioxidant capacity in rats exposed to different oxygen concentrations. **Methods.** Male Wistar rats were exposed to 14.4%, 20.9%, 35.5%, 39.8%, 62.5%, and 82.2% oxygen at 1 atmosphere absolute for 24 h. Serum levels of dROMs and BAP were examined by using a free radical and antioxidant potential determination device. The morphological characteristics of red blood cells were examined by phase contrast microscopy. **Results.** There were no differences in the levels of dROMs in rats exposed to 14.4%, 20.9%, and 35.5% oxygen. However, the levels of dROMs increased in the rats exposed to 39.8% and 62.5% oxygen. The levels of dROMs were the highest in the rats exposed to 82.2% oxygen. There were no differences in the levels of BAP with respect to the oxygen concentration. Morphological changes in the red blood cells induced by oxidative attack from reactive oxygen species were observed in the rats exposed to 39.8%, 62.5%, and 82.2% oxygen. **Conclusion.** Our results suggest that exposure to oxygen concentrations higher than 40% for 24 h induces excessive levels of oxidative stress in rats.

1. Introduction

Supplemental oxygen is used in treatment and as a countermeasure for acute and chronic diseases. When pilots of unpressurized aircrafts fly to areas at high altitudes, when climbers ascend high-altitude peaks and outpace their ability to acclimatize, or when divers inhaling compressed air return to the surface, the external pressure on the body decreases and the dissolved inert gases come out of solution in the form of bubbles in the body on depressurization [1, 2]. The resulting decompression sicknesses and air embolisms are initially treated by inhalation of oxygen-enriched air or exposure to mild hyperbaric oxygen at 1.25 atmospheres absolute (ATA) until hyperbaric oxygen therapy (100% oxygen delivered at 2-3 ATA) is administered [3-6]. Hypoxic or breathless patients with chronically obstructive pulmonary disease (COPD), who have low levels of oxygen in their blood, require oxygen at concentrations greater than that in

room air to achieve arterial oxygen saturations between 88% and 92% [7].

Oxygen therapy with or without pressure is associated with the risk of oxygen toxicity and excessive oxidative stress. Oxidative stress plays a key role in the pathogenesis of many diseases and their complications; the generation of free radicals and increased levels of oxidative stress are associated with atherosclerosis, cataract, retinopathy, myocardial infarction, hypertension, diabetes, renal failure, and uremia [8-10]. However, there are no data available on changes in the oxidative stress level and antioxidant capacity after exposure to different concentrations of oxygen. The analytical measurement of oxidative stress markers has been difficult because of the short half-life and high reactivity of the majority of reactive oxygen species and the applicability of measurement methods [11]. Blood samples are the appropriate biological materials for assessing the status of oxidants and antioxidants. A unique system for the

evaluation of oxidative stress levels and antioxidant capacity in the blood has been developed [12]. This evaluation approach is based on the free radical analytical system that mainly analyzes lipid hydroperoxides, which are relatively stable in the blood. This system has been used for both animal and human sera, which confirms its applicability [13–15].

In this study, we examined the derivatives of reactive oxygen metabolites (dROMs) as an index of oxidative stress levels (oxidant capacity) and the biochemical antioxidant potential (BAP) as an index of antioxidant capacity in rats exposed to different concentrations of oxygen at 1 ATA for 24 h.

2. Materials and Methods

2.1. Experimental Animals. All experimental and animal care procedures were performed in accordance with the guidelines stated in the Guide for the Care and Use of Laboratory Animals issued by the Institutional Animal Experiment Committee of Kyoto University (Kyoto, Japan).

2.2. Exposure to Different Concentrations of Oxygen. Ten-week-old male Wistar rats weighing between 200 g and 226 g were divided into 6 groups ($n = 5$ for each group). The individual groups were exposed to air containing low or high concentration of oxygen in a chamber ($75 \times 130 \times 85$ cm) at 1 ATA for 24 h by using a low-oxygen inhaler (Terucom Corp., Yokohama, Japan) or an oxygen concentrator (Ikiken Corp., Sayama, Japan), respectively. When the air chamber contained less or more than 20.9% oxygen, air with 2 different concentrations of oxygen from 2 tubes was transported to the aspirator: one tube contained normal air (20.9% oxygen) and the other tube had air containing 13% or 84% oxygen, which was procured from the low-oxygen inhaler or the oxygen concentrator, respectively. Then, the aspirator pumped mixed air into the chamber at the rate of 1 L/min. The low or high oxygen concentration in the chamber was adjusted by separately regulating the air flow from these 2 tubes. Normal air was transported by only 1 tube, while the air containing 20.9% oxygen was retained in the chamber. The oxygen concentration in the chamber was determined by using an oxygen monitor (Max O₂+AE; Maxtec Inc., UT, USA) attached to the chamber. The rats were maintained in individual, uniformly sized standard cages ($30 \times 40 \times 20$ cm) in the chamber. The room was maintained at $22 \pm 2^\circ\text{C}$ with 45%–55% relative humidity. Food and water were provided *ad libitum*.

2.3. Measurements of dROMs and BAP. The levels of dROMs and BAP were determined after the rats were exposed to different concentrations of oxygen. Blood samples were obtained from the tail of fully conscious rats and evaluated photometrically. A free radical and antioxidant potential determination device (Free Radical Analytical System 4; Health & Diagnostics, Grosseto, Italy) was used to automatically measure the levels of dROMs and BAP.

The dROMs are used as an index to determine the level of oxidative stress (oxidant capacity) by measuring the amount of organic hydroperoxide (ROOH) converted into radicals that oxidize *N,N*-diethyl-*p*-phenylenediamine [12, 16]. The levels of dROMs were expressed in Carr units (1 U·Carr corresponds to 0.08 mg hydroperoxide/100 mL H₂O₂). The BAP is used as an index to determine the biological antioxidant capacity and is measured on the basis of the capacity of the plasma sample to reduce ferric ions to ferrous ions. After blood samples were obtained, the rats were killed by intraperitoneal overdose of sodium pentobarbital.

2.4. Red Blood Cell Morphology. Using blood samples, the morphological profiles of red blood cells were observed by phase contrast microscopy (Nikon 80iF-PH-15; Tokyo, Japan).

2.5. Statistics. Means and standard deviations were calculated from the individual values by using standard procedures. One-way analysis of variance (ANOVA) was used to evaluate the mean differences among the 6 groups. When ANOVA analyses revealed significant differences in mean values, the groups were further compared using Scheffé's *post hoc* tests. A probability level of 0.05 was considered significant.

3. Results

The levels of dROMs in rats exposed to 39.8% and 62.5% oxygen were higher than those in the rats exposed to 14.4%, 20.9%, and 35.5% oxygen (Figure 1(a)). The level of dROMs in the rats exposed to 82.2% oxygen was the highest among the 6 groups. There were no differences in the levels of BAP among the 6 groups (Figure 1(b)). Morphological changes in red blood cells were observed in the rats exposed to 39.8%, 62.5%, and 82.2% oxygen (Figure 2).

4. Discussion

4.1. Exposure to Low Concentration of Oxygen. Acclimatization at high altitude results in changes in the respiratory, cardiovascular, and hematologic systems, which enhance oxygen delivery to the cells and tissues [17]. Decompression sickness occurs between initial hypoxic conditions and the onset of acclimatization, and the incidence and severity of the sickness depend on the rate of ascent, the altitude attained, and physiological susceptibility of individuals [5].

There is little data available regarding the oxidative stress level under low concentrations of oxygen. In this study, we examined the oxidative stress levels in rats exposed to low concentrations of oxygen. In rats exposed to 14.4% oxygen for 24 h, which is equivalent to the oxygen concentration at 3500 m/11500 feet altitude, no change was observed in the oxidative stress level (Figure 1(a)). Therefore, we conclude that low concentrations of oxygen do not induce excessive oxidative stress.

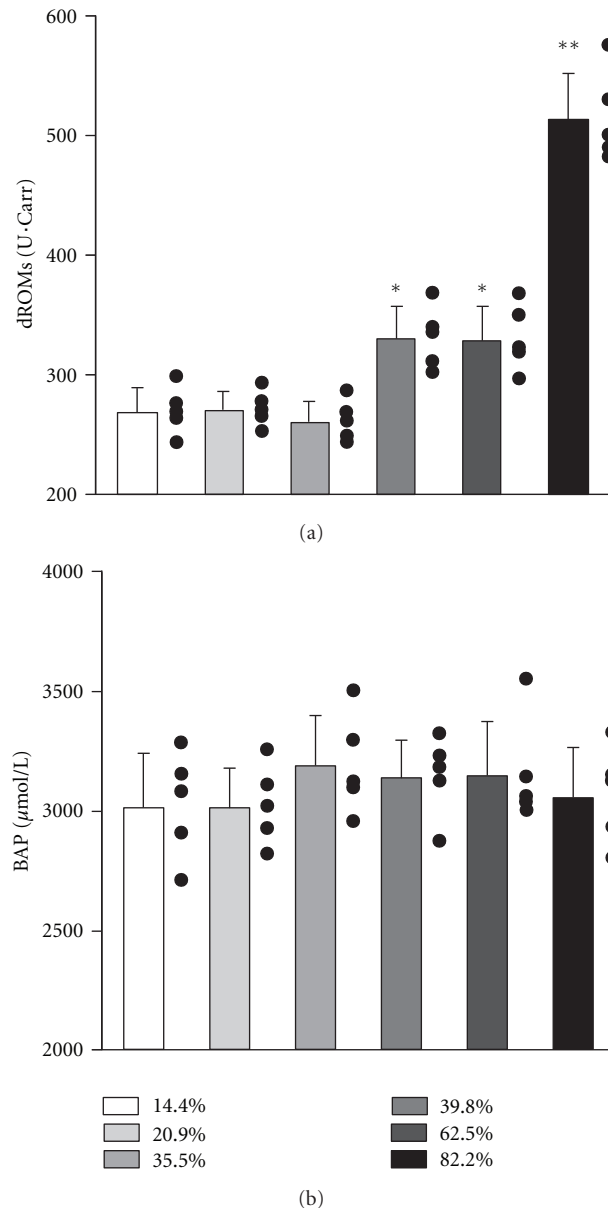


FIGURE 1: Levels of derivatives of reactive oxygen metabolites (a) and biochemical antioxidant potential (b) of rats after exposure to different concentrations of oxygen for 24 h. Values are expressed as the mean and standard deviation ($n = 5$ for each group). Five dots on the right side of the bar are the individual values of rats in each group. dROMs: derivatives of reactive oxygen metabolites; BAP: biochemical antioxidant potential. * $P < 0.05$ compared with values of 14.4%, 20.9%, and 35.5% oxygen; ** $P < 0.05$ compared with values of 14.4%, 20.9%, 35.5%, 39.8%, and 62.5% oxygen.

4.2. Exposure to High Concentration of Oxygen. Hyperbaric oxygen at 2–3 ATA with 100% oxygen induces acute changes, such as increased blood pressure and further reduction in heart rate [18], and causes chronic diseases like cataract formation [19–22]; hyperbaric oxygen is generally safe when pressures do not exceed 3 ATA and the length of treatment is less than 120 min [23, 24]. However, hyperbaric oxygen has

been reported to increase the levels of reactive oxygen species [25–27].

Patients with COPD inhale high concentrations of oxygen, generally up to 50% oxygen (fraction of inspired oxygen, FIO_2) when the partial pressure of oxygen in the arterial blood (PaO_2) is below 55 mmHg; these patients occasionally exhibit voluntary respiration failure, consciousness disturbance, and atelectasis when inhaling high concentrations of oxygen.

Oxygen therapy with or without pressure might induce excessive levels of oxidative stress. Excessive levels of oxidative stress are associated with many diseases, including atherosclerosis, cataract, retinopathy, myocardial infarction, hypertension, renal failure, and uremia [8–10]. In this study, we examined the oxidative stress levels in rats exposed to high concentrations of oxygen.

No change in the oxidative stress level was observed at 35.5% oxygen for 24 h (Figure 1(a)). We previously examined the effects of mild hyperbaric oxygen at 1.25 ATA with 36% oxygen on the neuromuscular system, including spinal motoneurons and their innervating muscle fibers [28, 29], type 2 diabetes [30–33], hypertension [34], type II collagen-induced arthritis [35], age-related decline in muscle oxidative capacity [36], and diabetes-induced cataracts [37] in mice and rats. Therefore, the data observed in this study suggest that mild hyperbaric oxygen at 1.25 ATA with 36% oxygen is effective for the inhibition and improvement of many metabolic diseases [28–37], without producing excessive levels of oxidative stress.

In contrast, 39.8% and 62.5% oxygen for 24 h induced excessive levels of oxidative stress, and the level of dROMs was the highest at 82.2% oxygen (Figure 1(a)). Oxidative stress level increases when the production of reactive oxygen species is markedly greater than the intrinsic antioxidant defenses. Patients with COPD inhale high concentrations of oxygen, and the possibility of accumulating excessive levels of oxidative stress will be greater when the inhaled oxygen concentration (FIO_2) is high. Therefore, we conclude that exposure to 40% oxygen for 24 h is a threshold for inducing an excessive level of oxidative stress.

In our previous study [15], we observed that obese rats with metabolic syndrome accompanied by insulin resistance, impaired glucose metabolism, and dyslipidemia had lower levels of BAP than normal Wistar rats. In addition, we observed that mild hyperbaric exposure at 1.25 ATA with 36% oxygen improves the levels of BAP in rats with hypertension [34]. These studies [15, 34] suggest that changes in the levels of BAP are reflected as an index of antioxidant capacity. In this study, we expected that exposure to higher concentrations of oxygen would decrease the levels of BAP compared with exposure to 20.9% oxygen because the levels of dROMs were increased by exposure to 39.8%, 62.5%, and 82.2% oxygen (Figure 1(a)). However, there was no difference in the levels of BAP among different concentrations of oxygen (Figure 1(b)). These results suggest that the antioxidant capacity is not affected by both low and high concentrations of oxygen, and thus, the antioxidant capacity did not change, at least after 24 h. However, we did not examine antioxidant enzyme levels of rats exposed to different concentrations

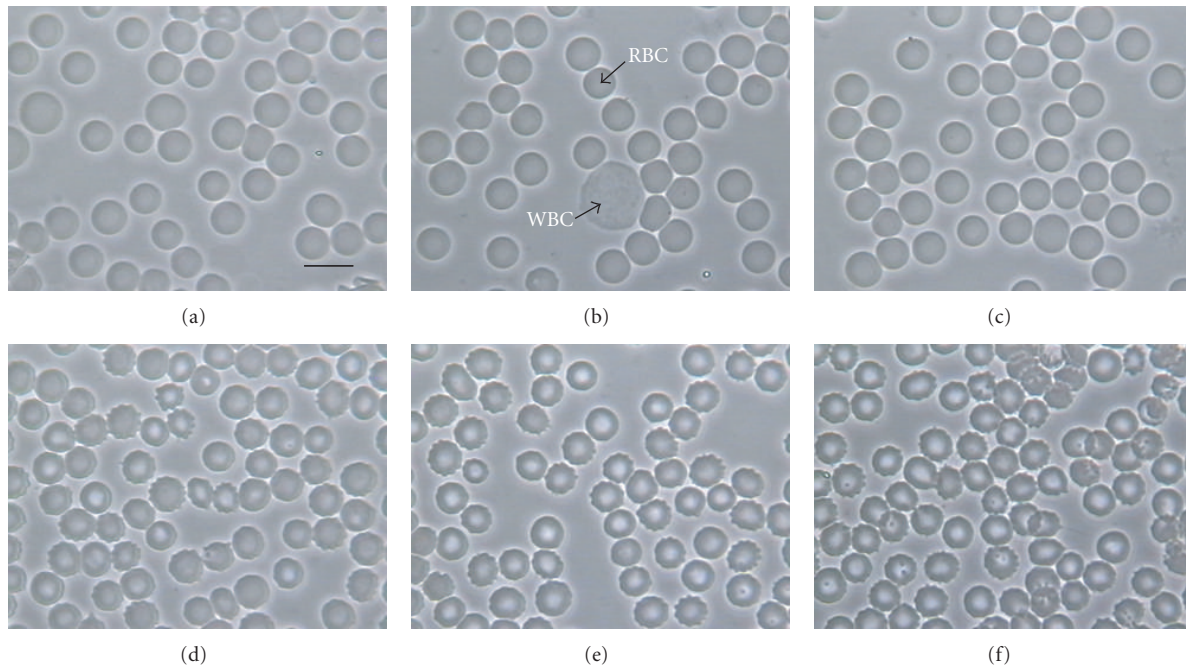


FIGURE 2: Morphological features of red blood cells after 24 h of exposure to different concentrations of oxygen: (a) 14.4%, (b) 20.9%, (c) 35.5%, (d) 39.8%, (e) 62.5%, and (f) 82.2%. RBC: red blood cell; WBC: white blood cell. Scale bar is 10 μ m.

of oxygen. In our subsequent study, we plan to examine the levels of BAP and antioxidant enzymes, for example, superoxide dismutase, catalase, and glutathione peroxidase, in rats exposed to different concentrations of oxygen for more than 24 h.

Transformed red blood cells, which were induced by oxidative attack from reactive oxygen species, were observed by exposure to 39.8%, 62.5%, and 82.2% oxygen (Figure 2); these findings are consistent with the increased levels of dROMs at these oxygen concentrations (Figure 1(a)). Therefore, we conclude that morphological changes in the red blood cells are linked to increased levels of oxidative stress.

5. Conclusion

Exposure to oxygen concentrations higher than 40% for 24 h induces excessive levels of oxidative stress.

Conflict of Interests

The authors declare that they have no conflict of interests.

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